A PLATE METHOD FOR ISOLATING ANAEROBES

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Methods previously described for plating anaerobes have involved the use of an anaerobic chamber or the inversion of the bottom of the Petri dish in the top and sealing with paraffin. The anaerobic chamber is not always available and is cumbersome. The inverted plate method is simple, but the plate, once opened, cannot be sealed again, and in opening, the agar frequently breaks, part clinging to the bottom and part to the top of the Petri dish. The layer of agar is also apt to slide over the glass so that there is danger of originally well isolated colonies becoming mixed. In the following method these difficulties are avoided.

Dilutions of the material to be plated are made in the usual way; melted deep agar tubes are inoculated and plates poured. The plates are immediately chilled and as soon as the agar sets, another deep tube of sterile agar is poured over the surface of each plate. The plates are again chilled to set the second layer of agar, and the surface of each is covered with a layer of melted paraffin about 2-3 mm. thick, care being taken to have the paraffin completely cover the surface of the agar. The second tube of agar should be cooled to 42 C. before it is poured over the surface of the plate. If it is used too hot it may kill the organisms in the first layer of agar. The paraffin is sterilized by heating to the smoking point in a shallow tin or aluminum cup. It is cooled until a film can be seen forming on the surface and then rapidly spread over the plates by a warm sterile spoon. Three or four ordinary teaspoonfuls cover the surface of a 100 mm. dish. The spoon is sterilized by flaming or by heating in the paraffin.

In order to open the plates, a thin section lifter is sterilized in the flame, cooled and used to lift the whole layer of paraffin; or blocks of paraffin may be cut out over the colonies to be examined without disturbing the rest of the plate. After the colonies are removed, the breaks in the paraffin covering may be sealed with more melted paraffin and the plates incubated longer. It is best to remove the colonies with sterile glass pipets drawn out to points that are not too fine. With care the top layer of agar may be lifted from the bottom layer, but this is usually not necessary.

Tetanus and gas bacilli grow well by this method. The tetanus bacillus colonies appear at the end of 24 hours' growth in dextrose agar as minute opaque points. At the end of 48 hours, they have grown into fleecy white, more or less spherical colonies about 2.5 mm. in diameter. If the plates are too thickly inoculated, the colonies of tetanus bacillus do not attain such size or acquire so much of the fleecy appearance.

Gas bacilli grown on blood-agar plates appear as small grayish colonies with a wide zone of hemolysis.

This method has been in use as a routine for 18 months, and there has been no difficulty in avoiding contaminations.